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Temporal and spatial variation in morphology and distributions of Central Texas lizards.

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Temporal and spatial variation in morphology and distributions of Central Texas lizards.

by

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Abstract

Urbanization has been recognized as an important factor influencing plasticity, evolution, and ecology in populations. However, responses to this novel anthropogenic pressure are taxon-specific and at times difficult to disentangle. A useful approach to elucidating these relationships is utilizing morphology given its influence over animal form and function in the environment. I investigated whether urbanization has caused morphology to change through time and across space in lizards in Central Texas. Utilizing a combination of measuring historical specimens and conducting modern field surveys, I found evidence of morphological variation at both temporal and spatial scales as a response to urbanization. Specifically, limb and toe measurements decreased through time in all five lizard species sampled and were larger on average in urban lizards. I also used modern sampling efforts to record presence-absence data to investigate whether urbanization has altered lizard distributions. I found evidence that distributions have potentially been affected by urban development in three lizard species. The implications of my study include a need for more systematic research on urban morphology that can begin in present day and the need to disentangle the interacting but potentially conflicting effects of plasticity, evolution, and ecology when making conclusions about urban and non-urban populations.

Introduction

Anthropogenic effects on biological systems have long been of interest to researchers, including in the fields of ecology (Browne et al. 2015; Dorresteijn et al. 2015; Ellis 2015), evolution (Orsini et al. 2012; Swaddle 2015), and conservation (Dyck 2012; Santamaría and Méndez 2012). More specifically, the impact of urbanization on these systems is of particular interest. Although global urban population growth has decreased, over half of the world's population currently lives in urban areas (Gao and O'Neill 2020). Additionally, a conservative estimate for global urban area growth from today through the year 2100 suggests an almost two-fold increase, and an increased pace of urbanization and land conversion worldwide (Gao and O'Neill 2020). Such novel and rapid alteration of the environment affects many facets of ecology and evolution as habitat is altered often beyond recognition from its previous state and populations face new challenges in cities. Scientists have therefore taken advantage of the unique opportunities presented by urban landscapes to study the eco-evolutionary effects of such environmental change on populations. A mechanistic approach allows for a broader understanding of the ecological and evolutionary principles that drive the differences we see between urban and non-urban populations that can be applied to a wider range of taxa.

The process of urban environments altering ecological mechanisms that in turn can lead to the evolution of populations can be mapped as a top-down approach. Urban environments cause changes in physical habitat structure, microclimates, resource availability, competition, and habitat productivity (Partecke 2014). These changes then influence ecological processes such as interactions with conspecifics, resource acquisition, phenology, and trophic relationships (Shochat et al. 2006). Altered ecological mechanisms then drive individuals to exhibit modified behavioral, morphological, and physiological traits compared to non-urban counterparts (Bowers and Breland 1996; Atwell et al. 2012; Bonier 2012). Selection can then act on these novel traits, leading to the adaptation of organisms to cities or the purging of lineages not well

suited for urban life (Shochat et al. 2006). Additionally, urbanization can alter existing selective pressures or introduce novel challenges that non-urban populations do not encounter. Environmental noise causing changes in bird song volume and frequency is a well-studied example of this (Brumm 2004; White 2020). Urbanization can also interact with other human-driven environmental alterations such as climate change. On land, increased temperature affects physiological traits by pushing organisms closer to their thermal maxima (Portner and Farrell 2008; Bennett et al. 2019). This particularly affects populations in warmer environments as they are already adapted to live close to their thermal maximum (Somero 2010; Pontes-da-Silva et al. 2018). In this respect, cities can actually act as a buffer against extreme temperature changes and provide more suitable habitat for some species (Parris and Hazell 2005). However, urbanization and climate change are often studied in isolation. Therefore, more mechanistic studies on how cities affect populations are needed to apply results to broader environmental principles.

This somewhat linear framework for viewing how ecological and evolutionary change occurs in urban centers is certainly limited, and studies often show the more complex nature of biological processes in these altered environments (Chamberlain et al. 2009). However, approaching responses to urbanization with a conceptual understanding of ecological and evolutionary mechanisms driving these changes is essential. Specific taxa and traits can then be studied with these principles in mind, and subsequent results applied to broader scientific understanding. One trait that is often tied to several biological mechanisms altered by urban environments is morphology.

Morphological traits are often associated with functions that allow individuals to respond to and persist in changing environments. Similarly, they are tied to many biological processes such as development, plasticity, and species diversification that can be applied in broader contexts. This makes morphology useful to study as a metric for organism response to environmental change. Notably, many morphological traits are plastic and can therefore change throughout an individual's life in response to environmental

variation (Pigliucci and Murren 2003). Therefore, the distinction between phenotypic variation in urban settings and changes in heritable traits implying evolution must be kept conscious when designing these studies (Lambert et al. 2021). Nonetheless, investigating the influence of urbanization on morphology is useful as a foundation for future work that could potentially make more concrete conclusions on the mechanisms and processes driving biological changes in cities. One taxonomic group that is often studied in these contexts is lizards due to their small size, relatively high abundance in both urban and non-urban habitats, and strong correlations between lizard morphology and function (Winchell et al. 2018a; Balakrishna et al. 2021).

Despite the utility of morphology, there is little consensus on how specific morphological characters change in response to urbanization, as responses are complex and taxon-specific. Body size is the most common morphological character analyzed in urbanization studies as it can inform various aspects of an animal's condition influenced by ecological variables such as resource availability, competition, and temperature differences. However, it is not understood why body sizes shift between urban and non-urban settings in some taxa or why there is no consistent directionality in these shifts. Urban lizards are generally larger in size than their non-urban counterparts, with one potential explanation being increased resource availability in cities (Thawley et al. 2019). However, perhaps better metrics of urban form and function in lizards are limb and toe sizes. Limb sizes in lizards correlate with a lizard's sprint speed, which in turn affects the ability for a lizard to capture prey and evade capture by predators (Losos 1990). Consequently, the differences in perch types and sizes and surface cover between urban and non-urban settings can affect limb size and sprint speed, as shown in *Anolis* lizards (Winchell et al. 2018b). These morphological differences have only been studied in a few lizard taxa, most commonly anoles, and the ecological and conservation implications of such shifts are still to be fully understood. Additionally, limb variation in urban-non-urban gradients appears to once again vary when different families are studied. Teiids and

dactyloids have both been shown to have increased limb and toe sizes in cities (Winchell et al. 2018b; Gómez-Benitez et al. 2020), whereas phrynosomatids such as the genus *Sceloporus* exhibit reduced limb and toe sizes in urban areas (Sparkman et al. 2018; Putman et al. 2019). Importantly, these varying results are not indicative of a lack of greater ecological mechanisms. Differences in morphological trends between families may indicate behaviors and life history traits that are a result of the diversification between these groups. However, such conclusions have yet to be made. Therefore, investigating morphological differences between urban and non-urban lizards of various families can expand this knowledge and potentially have conservation implications that are catered to each family's behaviors and environments.

The Edwards Plateau ecoregion of Central Texas provides an ideal opportunity to study the effects of urbanization on lizard populations due to the presence of a fairly large area of low development and natural areas bordered by highly urbanized centers. The region consists of sizeable areas of relatively undisturbed grasslands, mixed forest and woodlands with small urban centers interspersed throughout. However, along its eastern border lies one of the fastest growing urban regions in the country, the Austin-San Antonio corridor. Both of these cities are among the highest in population growth in the nation (US Census Bureau 2019). The current distributions of various lizard families in the region further support its utility for research. Analyzing all available records of five lizard species- *Sceloporus olivaceus, Anolis, Aspidoscelis gularis*, *Urosaurus ornatus*, and *Cophosaurus texanus*- uploaded to the citizen science platform iNaturalist showed that while all five species are commonly found across the Edwards Plateau, *Urosaurus ornatus* and *Cophosaurus texanus* do not range as far into the city of Austin (Figure 1; GBIF 2021). This could be due to differences between these lizards that allow some species to persist in and adapt to urban areas whereas others rely on less-disturbed land to thrive. This hypothesis is further supported by the lizards' varying life history traits (Figure 2). Of the genera present in the Edwards Plateau, studies on morphology in urban contexts have only been conducted on *Sceloporus*, *Anolis*, and *Aspidoscelis*; the lack

of consistent results between lizard families mentioned above invites more detailed research (Putman et al. 2019; Thawley et al. 2019; Gómez-Benitez et al. 2020).

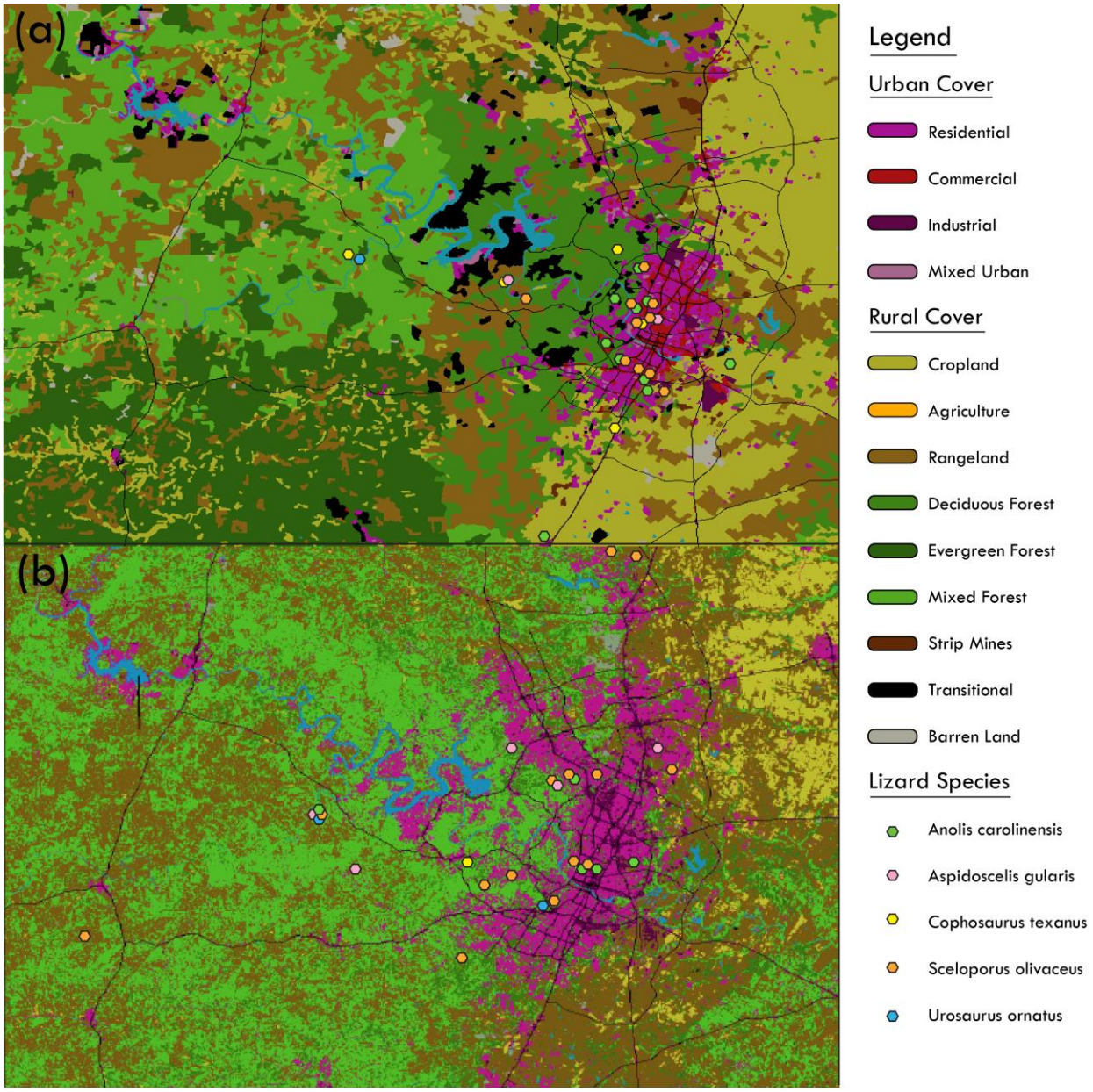
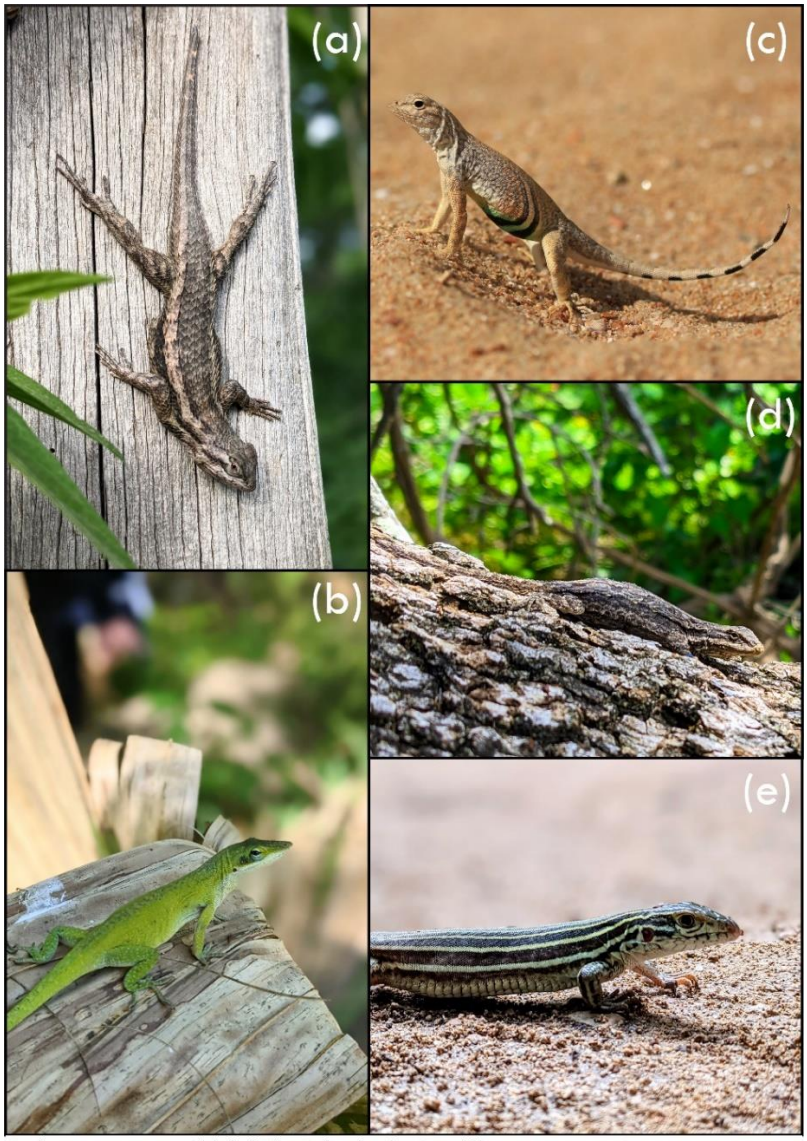


Figure 1 – Land cover in the Edward's Plateau region around Austin, Texas with partial lizard distributions. Lizard occurrences, shown by the dots, are actual specimens that were measured from historical collections and modern sampling efforts. (a) shows land cover for the 1970's-1990's (Price et al. 2009). Notably, urban cover increased in modern day as shown by the Texas Ecological Mapping System (b), and modern specimens of *Anolis carolinensis*, *Aspidoscelis gularis*, *Cophosaurus texanus*, and *Sceloporus olivaceus* were collected from land that became urbanized since the 1970's. The non-urban land west of Austin also became more homogenous from the 1970's to modern times, as (a) shows a more heterogenous mix of deciduous, evergreen, and mixed forest compared to the majority mixed forest and rangeland shown in (b).



Species	Adult size (SVL)	Preferred perch	CTmax (°C)	Sources
a - *Sceloporus olivaceus*	46 mm	Trees and posts	36	Davis 1967; Fitzpatrick et al. 1978
b - *Anolis carolinensis*	40 mm	Trees and bushes	41.28	Jenssen et al. 1995; Lailvaux and Irschick 2007
c - *Cophosaurus texanus*	47 mm	Terrestrial	42.1	Goldberg 2013; Osmanski 2014
d - *Urosaurus ornatus*	39 mm	Trees	43.99	Gilbert and Miles 2016; Martin 1973
e - *Aspidoscelis gularis*	52 mm	Terrestrial	41	Osmanski 2014; Ramirez-Bautista et al. 2009

Figure 2 – Lizard species photographed in the field in their most commonly encountered habitat. (a) *Sceloporus olivaceus*, the Texas spiny lizard, was the most common lizard and was present at six of the seven sites surveyed. They were typically encountered basking on open trunks and man-made wooden posts as shown. (b) *Anolis carolinensis*, the green anole, was typically found on low-lying shrubs, palm fronds, and trunks at urban sites. (c) *Cophosaurus texanus*, the greater earless lizard, was not seen during surveys, but is known to inhabit open areas with sandy-rocky soil and sparse vegetation. Image by Brittney A. White. (d) *Urosaurus ornatus*, the ornate tree lizard, was mainly observed on horizontal tree branches at various heights. (e) *Aspidoscelis gularis*, the common spotted whiptail, was typically seen in areas with open surfaces surrounded by grassy cover.

I designed a study to investigate morphological differences between urban and non-urban lizards in Central Texas. Building off of previous research, I identified nine morphological traits to measure that could correlate with lizard function in both urban and non-urban environments. I then selected five lizard species to measure: *Sceloporus olivaceus, Anolis carolinensis, Aspidoscelis gularis, Urosaurus ornatus,* and *Cophosaurus texanus*. These lizards were selected based on their abundance in the Edwards Plateau, records of systematic historical collection, their varied distribution across urban environments and environmental niches, and the simultaneous presence and absence of previous studies on morphological change. I measured all available specimens of these taxa in the Biodiversity Collections at UT Austin (formerly the Texas Natural History Collections) sampled as far back as 1940 in Travis County. These specimens, when collected systematically, offer snapshots of populations during snippets of time that can help discern how morphology has changed as urban development expands and support future work once traits are known to be heritable. Using the location data available for the specimens and both historical and modern land use data I identified sites to sample in the present day to measure the same traits in modern lizards in both urban and non-urban environments. I hypothesize that there will be morphological variation within lizard species across both temporal and spatial scales. Specifically, urban lizards will exhibit different average lengths in limb, toe, and body sizes compared to non-urban lizards with potential ecological implications. There will also be correlations between limb and toe lengths and environmental variables in modern times. Lizard morphology will also change through time as the region has become more urbanized. Additionally, I predict that lizard distributions will have changed through time as a result of urbanization. These results could help inform conservation management such as sustainable development and eco-friendly public spaces that are cognizant of the biological implications of urbanization. However, if morphology and distributions are not found to be different, this can nonetheless

inform future studies and allow conservation efforts to be focused on more threatened species that have already been shown to respond negatively to urban development.

Methods

Land Cover Quantification

In order to assess morphological differences between urban and non-urban lizards accurately, land cover for the Edwards Plateau must be accurate to the time period in which the lizard was collected. I acquired land cover data from a variety of sources and organized the data by decade: the USGS NGP for the 1940's-1960's (U.S. Department of the Interior 2017), the USGS water survey for the 1970's-1990's (Price et al. 2006), and the Texas Ecological Mapping System for the 2000's-present (Texas Parks and Wildlife 2015). I then categorized land cover as either urban or non-urban based on consistent labeling of urban land across maps. Finally, I further refined land cover according to categories denoted by the USGS water survey's map symbology (Price et al. 2006): urban land was categorized as commercial (CM), park (PK), residential (RS), transitional (TS), and university (UN). Non-urban land was categorized as cropland (CP), rangeland (RG), mixed forest (MF), preserve (PV), or deciduous forest (DF). Additional land cover from which lizards were not collected but was present in the maps were agricultural land, strip mines, barren land, evergreen forest, and industrial land. I imported all data into ArcGIS using the NAD 1983 geographic coordinate system. The results of land cover quantification are shown in Figure 1.

Historical Specimen Measurement

I obtained specimens of *Sceloporus olivaceus* (n=111), *Urosaurus ornatus* (n=245), *Anolis carolinensis* (n=55), *Cophosaurus texanus* (n=81), and *Aspidoscelis gularis* (n=17) from the Biodiversity Collections at the University of Texas to assess historical morphology. Individuals with poor or absent location information were not selected for measurement and analysis. Upon receiving specimens, the snout-to-vent length (SVL), head length, head width, distal forelimb and hindlimb length, proximal forelimb and hindlimb lengths, and the fourth toe length for both the forelimb and hindlimbs were measured (Figure 3).

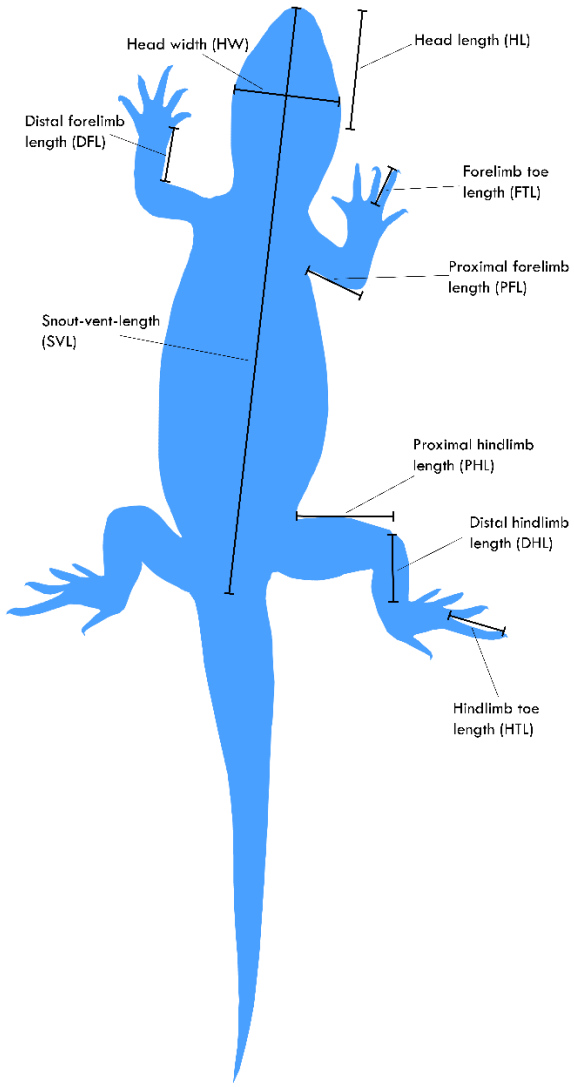


Figure 3 – Lizard outline graphic showing morphological measurements taken on both historical and modern specimens. Head length was taken from the snout to the end of the parietal. Head width was taken along the widest part of the head outlined by the postorbital bones behind the eyes. Proximal limb lengths were more variable than distal limb lengths as the intersections of limb and body had different levels of skin tension in each individual. All measurements were taken on the right side of specimens.

I did not measure specimens if they did not meet a specific threshold size that denoted adult and juvenile specimens (Figure 1). An initial twenty measurements were taken on five specimens in order to assess the replicability of measurements. Head length was taken in a straight dorsal line from the snout to the end of the parietal. Head width was taken on the postorbital bones right behind the eyes. Distal limb lengths

(between the radius/ulna and metatarsals and between the tibia/fibula and metacarpals) were taken using the elbow/knee as a constant reference point (Figure 3). Proximal limb lengths were also taken using the elbow/knee as a reference point but utilized the intersection between the humerus/femur and the main body as the second reference point. As such, proximal limb lengths were more difficult to measure due to differing levels of skin tension.

Modern Sampling of Lizards

I conducted modern sampling of lizards at field sites to supplement historical morphological measurements. Sites were chosen using known distribution records from both the historical records of specimens measured and iNaturalist data for each species. In total, 4 urban and 3 non-urban sites were sampled around the Austin area. The Shoal Creek greenbelt urban site (30.2922, -97.7506) is a commonly visited park near downtown Austin characterized by low-density oak and juniper growths along the edge of concrete trails and Shoal Creek. The University of Texas urban site (30.2849, -97.7337) supports a section of Waller Creek running through main campus with oak trees and low-lying shrubs and bushes. Oak View Park (30.4182, -97.7701) is an urban park of oak and juniper woodland with gravel paths running throughout. It is next to Caraway Elementary School and supports a playground in addition to the trails. Southwest Greenway (30.2893, -97.7024) is a recently built park designed to enhance green spaces in the Austin area. A mixture of shrubs, grassland, and sparse oak and juniper trees border a man-made pond across the park. Both the Barton Creek greenbelt (30.2485, -97.7965) and St. Edward's Park (30.4068, -97.7903) were similar non-urban sites within the Austin metropolitan area. These sites are characterized by a mixture of large patches of shrub grassland and mixed forest at lower altitudes and less dense juniper woodland and rocky outcrops at higher altitudes. While there are dirt paths for public use, they were not observed to disrupt the interconnectivity of habitat patches. Reservation requirements due to the COVID-19 pandemic restricted access to the final non-urban site, Hamilton Pool. Therefore, I

surveyed Reimer's Ranch (30.3691, -98.1295) instead. The county park is located approximately one mile north of Hamilton Pool and supports the same type of habitat-mixed forest along the Pedernales River with grassy open spaces and rocky outcrops. Like the other non-urban sites, dirt paths for human were not observed affecting the interconnectivity of habitat.

Sites were surveyed for a minimum of two days to a maximum of three days beginning in late February and ending in late May 2021 from roughly 10 AM to noon, when lizards are known to bask. The sites were surveyed extensively by walking along both man-made paths and through vegetation off-path to cover the most area. This also allowed for multiple microhabitats to be visited at various sites to ensure the highest probability of encountering the five lizard species. Lizards were captured using a telescoping fishing pole with a lasso honda knot at the end. The lasso tightened when placed around the lizard's neck or upper body and did not harm the animals. Upon capture the lizard was evaluated for its species-specific size threshold (Figure 2) and subsequently marked with non-toxic washable latex paint so as to prevent recapture during subsequent surveys. Lizards were marked on the bottom of their right rear foot so as to not interfere with mating behavior. Morphological measurements were then taken and recorded. Environmental variables were also measured when physically feasible including perch height, perch width, perch type, and perch surface temperature. All research was conducted under IACUC protocol # AUP-2020-00172.

Statistical Analysis

Before statistical analysis, I tested each sample group for normality through both visual methods and statistical tests. Visual methods were a combination of histograms and density curves. Shapiro-Wilk tests were then used to make the final determination. Morphological measurements were then corrected for size by dividing each individual measurement by the SVL of the corresponding specimen. Analyses were then conducted on the resulting ratios. Comparisons were made between average measurements of urban and

non-urban specimens across decade groups, between types of land cover within each decade group, and between perch types for modern lizards. Since only a few morphological characters out of the entire data set were found to be non-normally distributed, I ran non-parametric Kruskal-Wallis tests of significant variation between multiple groups to account for non-normal variables (Ostertagová et al. 2014). I then ran post-hoc Dunn's tests to verify between which groups the significant variation lay. Results were visualized with boxplots. I applied Bonferroni corrections to account for the same data being analyzed repeatedly. The value of n for the Bonferroni correction was taxon specific and depended on the number of comparisons made. Significant variation was defined as $p<0.05$. For the Results and Discussion, significant variation is referred to as "variation", whereas non-significant variation is referred to as "trend". For modern lizard measurements, regression analyses were conducted to visualize any relationship between average trait sizes and perch height.

	Sample Size							
	1940-1960		1970-1990		2000-2010		2020	
Species	**Urban**	**Non-Urban**	**Urban**	**Non-Urban**	**Urban**	**Non-Urban**	**Urban**	**Non-Urban**
Anolis carolinensis	8	5	21	10	2	3	14	0
Aspidoscelis gularis	4	0	4	1	1	6	1	3
Cophosaurus texanus	13	27	16	22	0	3	0	0
Sceloporus olivaceus	26	14	11	16	17	8	10	6
Urosaurus ornatus	4	6	1	216	0	4	0	1

Table 1 – Sample sizes for all five lizard species in both urban and non-urban environments across decade groups. *Sceloporus olivaceus* had the most consistent sample size through time and between sites. While *Urosaurus ornatus* had the largest total sample size this was overwhelmingly represented by non-urban individuals from the 1970-1990's. *Urosaurus* also had the smallest number of urban individuals.

Results

Morphological Variation Through Time

Analyzing morphology through time showed significant variation between decade groups. Table 2 displays *p*-values for temporal variation and trends in lizard morphology in urban settings, whereas Table 3 does the same for non-urban sites. Bonferroni correction of *p*-values depended on the number of comparisons being made. In order to simplify results, all morphological character measurements and values refer to the ratio of the respective morphological character to the SVL unless otherwise specified. Urban specimens of *Sceloporus olivaceus* (n=64) showed variation in four morphological characters. Proximal forelimb length decreased from both the 1940-1960's and the 2000-2010's through the modern decade. Proximal hindlimb length only decreased from the 1970-1990's to 2021. Forelimb toe length decreased from both the 1940-1960's and the 1970-1990's to modern day (Figure 4). SVL initially increased from both the 1940-1960's and the 1970-1990's through the 2000-2010's. However, body size decreased in modern day. Non-urban specimens of *S. olivaceus* (n=44) exhibited variation in distal limb lengths and hindlimb toe lengths. Distal forelimb length and hindlimb toe length increased from the 2000-2010's to 2021, whereas distal hindlimb length decreased from the 1940-1960's to the 2000-2010's (Figure 4).

	Measurement								
Species	SVL	HL	HW	PFL	DFL	FTL	PHL	DHL	HTL
Anolis carolinensis									
1940-1960:1970-1990	0.127	0.735	0.167	0.229	0.528	0.932	0.8	0.4	0.26
1940-1960:2000-2010	0.647	0.515	0.367	0.828	0.745	0.696	1	0.815	0.506
1940-1960:2020	0.552	0.745	0.284	0.82	0.242	**4.68E-03**	0.376	4.05E-02	0.356
1970-1990:2000-2010	0.179	0.614	0.85	0.657	0.994	0.712	0.883	0.821	0.938
1970-1990:2020	0.284	0.992	0.773	8.20E-02	0.459	**2.47E-04**	1.55E-01	0.111	0.88
2000-2010:2020	0.408	0.625	0.752	0.719	0.73	3.62E-02	0.604	0.34	0.884
Aspidoscelis gularis									
1940-1960:1970-1990	**7.24E-03**	0.35	0.815	0.243	0.35	0.35	0.726	0.815	0.102
Cophosaurus texanus									
1940-1960:1970-1990	0.861	**4.37E-03**	0.188	0.861	0.965	0.539	0.313	8.72E-02	0.33
Sceloporus olivaceus									
1940-1960:1970-1990	0.413	0.265	2.00E-01	4.50E-01	0.928	0.804	0.393	0.898	0.329
1940-1960:2000-2010	**8.96E-04**	0.423	0.469	0.71	4.04E-02	0.269	0.817	0.497	7.64E-02
1940-1960:2020	0.217	0.938	2.62E-02	**1.31E-03**	0.79	**1.08E-03**	1.44E-02	0.392	0.55
1970-1990:2000-2010	**5.89E-04**	0.093	0.543	0.677	8.24E-02	0.262	0.532	0.668	1.88E-02
1970-1990:2020	0.705	0.325	0.403	3.96E-02	0.763	**2.60E-03**	**6.92E-03**	0.404	0.768
2000-2010:2020	**1.75E-04**	0.579	0.131	**6.43E-03**	0.175	2.57E-02	1.36E-02	0.183	4.97E-02

Decade group

Table 2 – P-values for Kruskal-Wallis and Dunn's tests conducted on morphological traits in four of the five lizard species through time in urban sites. Significant values are in bold, determined after Bonferroni correction. Bonferroni values were determined by the number of temporal comparisons made per species: 6 for both *Anolis carolinensis* and *Sceloporus olivaceus* and one for *Aspidoscelis gularis* and *Cophosaurus texanus*. There were six time period ranges compared: between the 1940-1960's and the 1970-1990's, between the 1940-1960's and the 2000-2010's, between the 1940-1960's and the 2020's, between the 1970-1990's and the 2000-2010's, between the 1970-1990's and the 2020's, and between the 2000-2010's and the 2020's. Significant values do not indicate whether average measurements increased or decreased between decade groups.

		Measurement								
	Species	**SVL**	**HL**	**HW**	**PFL**	**DFL**	**PHL**	**DHL**	**FTL**	**HTL**
Decade group	***Anolis carolinensis***									
	1940-1960:1970-1990	2.19E-02	0.516	0.891	0.784	0.108	0.864	0.432	0.218	0.973
	1940-1960:2000-2010	0.231	0.945	0.297	0.483	0.657	0.561	0.516	0.166	0.707
	1970-1990:2000-2010	0.563	0.642	0.297	0.582	0.399	0.431	0.947	0.609	0.697
	Aspidoscelis gularis									
	2000-2010:2020	0.876	0.243	**2.40E-02**	0.102	0.243	0.35	0.243	0.697	0.947
	Cophosaurus texanus									
	1940-1960:1970-1990	0.133	**1.72E-03**	0.544	0.95	0.399	0.392	0.134	0.3	0.779
	1940-1960:2000-2010	0.736	0.341	6.65E-02	**9.27E-03**	**8.94E-03**	0.782	0.534	0.404	0.605
	1970-1990:2000-2010	0.714	0.602	0.126	**1.10E-02**	2.84E-02	0.9	0.189	0.19	0.708
	Sceloporus olivaceus									
	1940-1960:1970-1990	0.574	0.267	0.337	3.52E-02	0.147	0.619	0.62	0.289	0.696
	1940-1960:2000-2010	1.25E-01	0.848	0.406	0.763	1.16E-02	0.428	**7.17E-03**	0.164	1.02E-01
	1940-1960:2020	0.137	8.88E-01	0.182	1.82E-01	0.489	3.38E-02	0.443	0.748	4.79E-02
	1970-1990:2000-2010	4.08E-02	4.58E-01	9.63E-02	0.141	0.174	0.696	1.96E-02	0.597	0.179
	1970-1990:2020	0.278	0.481	3.63E-02	0.883	6.99E-02	7.14E-02	0.67	0.666	2.20E-02
	2000-2010:2020	9.30E-03	9.76E-01	0.601	3.25E-01	**7.00E-03**	1.86E-01	0.165	0.43	**2.07E-03**
	Urosaurus ornatus									
	1940-1960:1970-1990	4.53E-01	**2.97E-04**	**1.94E-04**	0.857	0.26	5.18E-02	1.00E-01	0.606	6.80E-02
	1940-1960:2000-2010	0.641	**4.62E-03**	1.88E-01	0.743	0.933	0.581	0.96	0.798	0.861
	1970-1990:2000-2010	0.227	5.12E-01	1.69E-01	0.756	0.302	0.374	0.199	0.903	0.16

Table 3 - P-values for Kruskal-Wallis and Dunn's tests conducted on morphological traits for five lizard species through time in rural sites. Significant values are in bold, determined after Bonferroni correction. Bonferroni values were determined by the number of temporal comparisons made per species: 6 for *Sceloporus olivaceus*, 3 for *Anolis carolinensis*, *Aspidoscelis gularis*, and *Cophosaurus texanus*, and one for *Aspidoscelis gularis*. There were six time period ranges compared: between the 1940-1960's and the 1970-1990's, between the 1940-1960's and the 2000-2010's, between the 1940-1960's and the 2020's, between the 1970-1990's and the 2000-2010's, between the 1970-1990's and the 2020's, and between the 2000-2010's and the 2020's. Significant values do not indicate whether average measurements increased or decreased between decade groups.

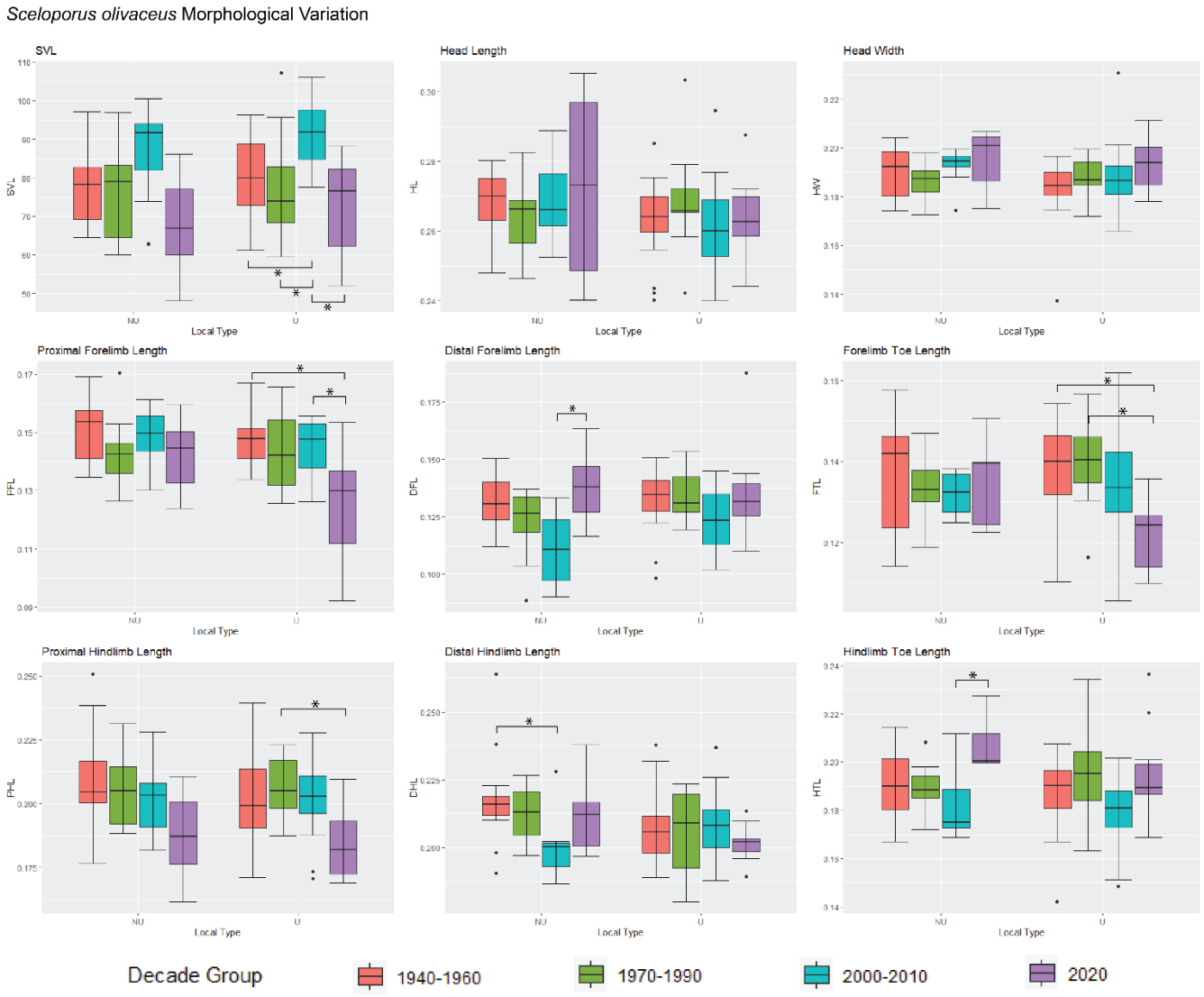


Figure 4 – Morphological variation through time for *Sceloporus olivaceus*. Asterisks denote statistical significance determined through Kruskal-Wallis and Dunn's tests and defined as p<8.33E-3 after Bonferroni correction. Significant variation was found in 7 morphological characters. In urban lizards SVL, proximal forelimb length, forelimb toe length, and proximal hindlimb length decreased through time. In rural lizards distal forelimb and hindlimb length and hindlimb toe length varied. Distal forelimb length increased from the 2000-2010's through 2021. Distal hindlimb length decreased from the 1940-1960's to the 1970-1990's. Hindlimb toe length then increased from the 2000-2010's to modern day.

Only forelimb toe length varied through time in urban *Anolis carolinensis* (n=45), increasing from both the 1940-1960's and the 1970-1990's to 2021 (Figure 5). There was no temporal variation in rural *A. carolinensis*. Urban specimens of *Urosaurus ornatus* (n=4) were too few to compare through time. In non-urban specimens of *U. ornatus* (n=226) head sizes varied through time. Head length increased through

time, from the 1940-1960's through both the 1970-1990's and the 2000-2010's. Conversely, head width decreased from the 1940-1960's to the 1970-1990's (Figure 6).

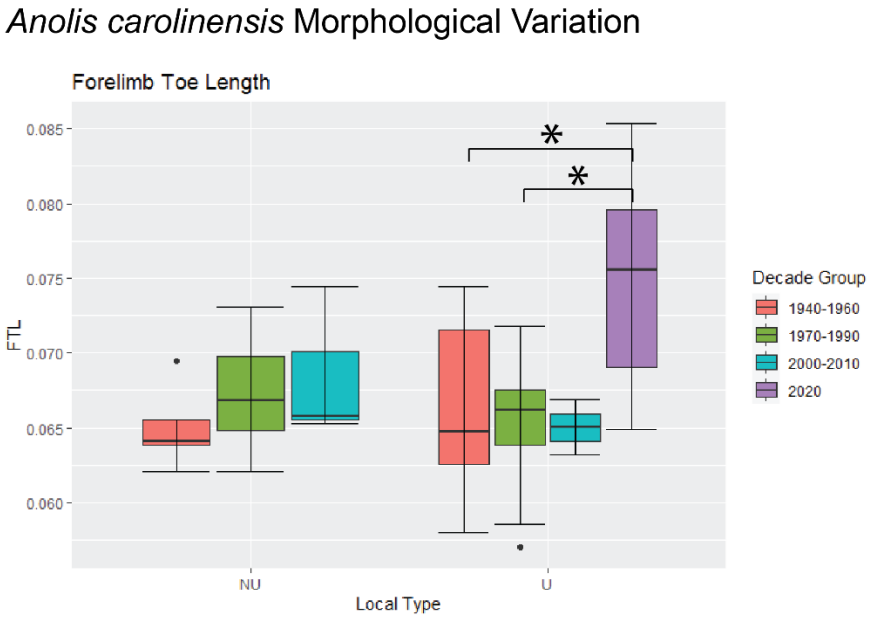


Figure 5 – Morphological variation through time in both urban and non-urban settings for *Anolis carolinensis*. Asterisks denote statistical significance determined through Kruskal-Wallis and Dunn's tests and defined as $p<1.67E-2$ after Bonferroni correction. Only forelimb toe length varied through time in urban sites, increasing from both the 1940-1960's and the 1970-1990's through modern day.

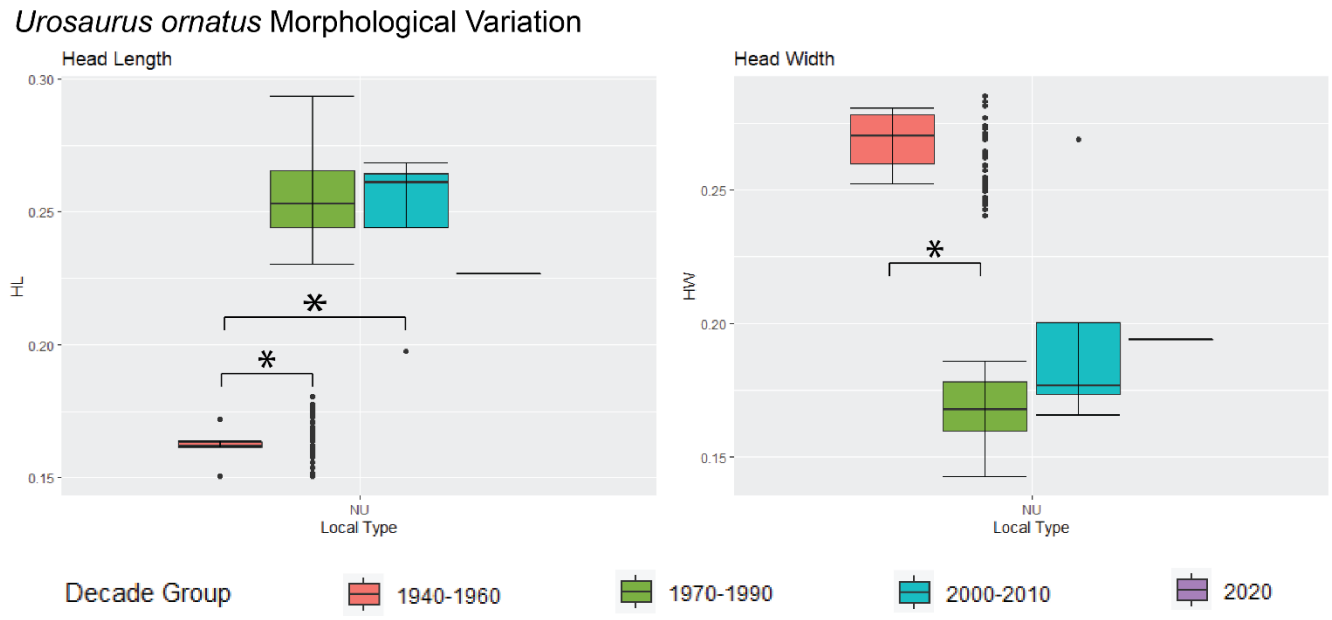


Figure 6 – Morphological variation through time in both urban and non-urban settings for *Urosaurus ornatus*. Asterisks denote statistical significance determined through Kruskal-Wallis and Dunn's tests and defined as p<1.67E-2 after Bonferroni correction. All variation was found in non-urban environments; comparisons were not made in urban environments due to small sample sizes. Head length and head width both varied from the 1940-1960's to the 1970-1990's, but while head length increased head width decreased. Head length also increased from the 1940-1960's to the 2000-2010's.

Specimens of *Aspidoscelis gularis* only displayed temporal variation in one trait. Urban SVL decreased from the 1940-1960's to the 1970-1990's (Figure 7). Urban specimens of *Cophosaurus texanus* (n=29) showed an increase in head length from the 1940-1960's to the 1970-1990's (Figure 8). Three characters varied in non-urban specimens of *Cophosaurus* specimens (n=52). Proximal and distal forelimb lengths decreased from historical specimens to the 2000-2010's, and head length increased from the 1940-1960's to the 1970-1990's (Figure 8).

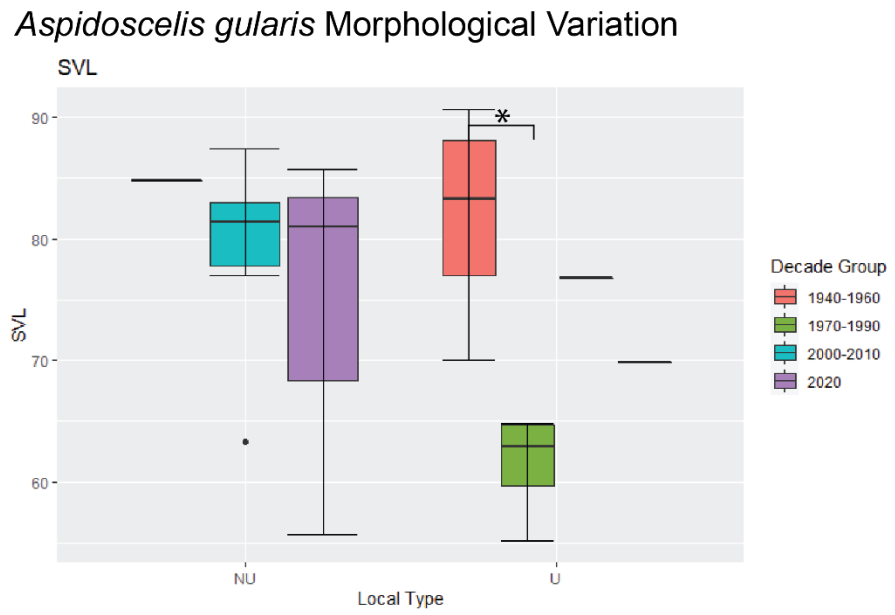


Figure 7 – Morphological variation through time for *Aspidoscelis gularis*. Asterisks denote statistical significance determined through Kruskal-Wallis and Dunn's tests and defined as $p<0.05$. Only SVL varied temporally, decreasing from the 1940-1960's to the 1970-1990's in urban lizards.

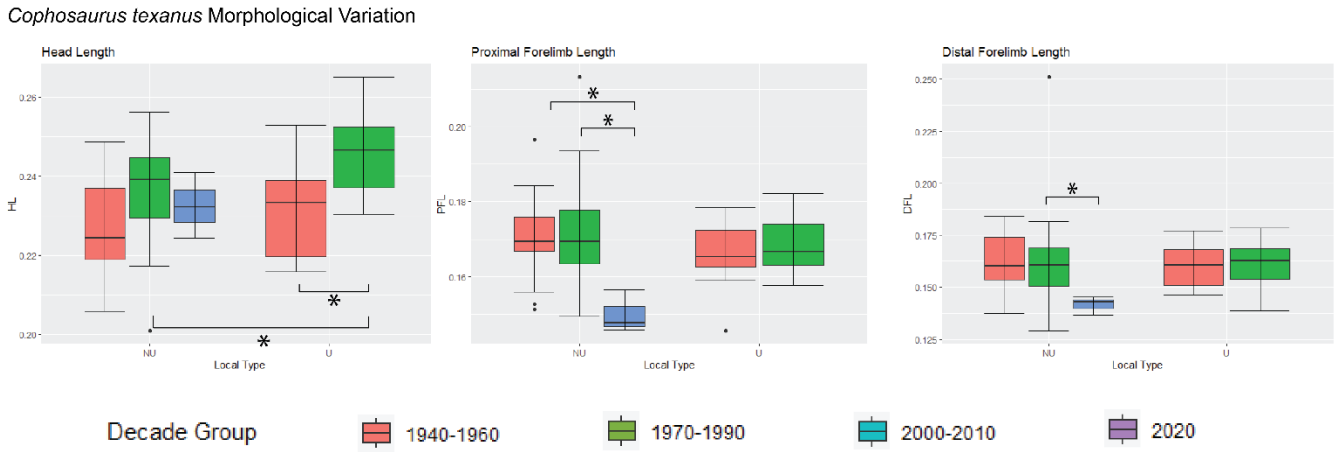


Figure 8 - Morphological variation through time and between urban and non-urban specimens for *Cophosaurus texanus*. Asterisks denote statistical significance determined through Kruskal-Wallis and Dunn's tests. Significance was defined as $p<1.67E-2$ for temporal variation after Bonferroni correction and $p<0.05$ for spatial variation. Head length increased from the 1940-1960's to the 1970-1990's in urban lizards. Head length overall was also larger in urban lizards than in those from non-urban sites. Proximal and distal forelimb lengths decreased from both the 1940-1960's and the 1970-1990's to the 2000-2010's.

Urban and Non-urban Morphology

Morphology between urban and non-urban lizards varied in historical specimens but not modern individuals. Within each decade group, morphological trends were recorded at two different resolutions of land cover: a more general categorization of urban and non-urban, and then further refinement of the sites using the USGS water survey land categories (Price et al. 2006). *Sceloporus olivaceus* collected from the 1940's-1960's showed smaller distal hindlimb lengths in urban lizards (Figure 9). When habitat was further refined using USGS terminology (Appendix 1), five morphological traits varied: head length, head width, proximal and distal forelimb length, and distal hindlimb length (Appendix 2). All characters had smaller average values in the urban habitat types: commercial, park, residential, and university. However, head length was larger in mixed forest individuals than commercial lizards. Head width and proximal forelimb length also varied between non-urban sites, with measurements from mixed forest and rangeland smaller than those from cropland (Appendix 2). Specimens of *S. olivaceus* collected from the 1970's-1990's varied at the refined level in proximal forelimb length, distal hindlimb length, and hindlimb toe length. Proximal forelimb lengths were smaller in park sites compared to commercial, preserve, and residential sites. Distal hindlimb lengths were smaller in park and rangeland sites compared to mixed forest sites. Hindlimb toe lengths were smaller in preserve sites compared to park and rangeland sites. In the 2000's-2010's, proximal and distal forelimb length, distal hindlimb length, and hindlimb toe length varied across land categories. Proximal forelimb lengths were smaller in preserve sites than in mixed forest and residential sites. Distal forelimb and hindlimb length were both smaller in park than commercial sites, and distal forelimb length was also smaller in mixed forest and preserve sites than commercial sites. Hindlimb toe length was smaller in rangeland than mixed forest sites. *Sceloporus olivaceus* measured in modern surveys did not vary between urban and non-urban lizards. Since *Sceloporus* were only present at two types of sites, mixed forest and parks, site refinement was not necessary.

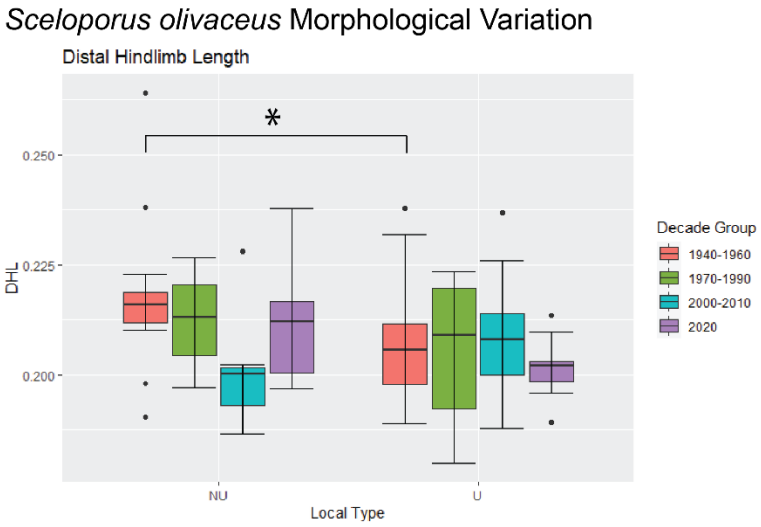


Figure 9 – Morphological variation in *Sceloporus olivaceus* between urban and non-urban sites for each decade group. Asterisks denote statistical significance determined through Kruskal-Wallis and Dunn's tests and defined as p<8.33E-3 after Bonferroni correction. Only distal hindlimb length was found to vary between urban and non-urban sites. Distal hindlimb lengths were smaller in urban individuals of the 1940-1960's.

Specimens of *Anolis carolinensis* collected in the 1940's-1960's varied in four traits across multiple refined urban and non-urban sites (Appendix 3). Head length and width were smaller in commercial sites than in park and rangeland sites, respectively. Both distal forelimb and hindlimb lengths varied, with forelimb length smaller in commercial than in park sites and hindlimb length smaller in deciduous forest sites (DF) than in parks. In the 1970's-1990's, head width was highly variable, being smaller in parks than in both rangeland and residential sites. Lizards from preserves and transitional sites also had more narrow heads than those in rangeland. All limb lengths varied in this decade group. While proximal forelimb lengths were larger in parks than in residential sites and deciduous forest, distal forelimb length was smaller in parks than transitional sites. Proximal and distal hindlimb lengths were larger in deciduous forest than in rangeland and parks, rangeland, residential, and transitional sites, respectively. The sample size of anoles collected in the 2000's-2010's (n=5) was too small to make meaningful comparisons of morphology between sites. Anoles were only captured in urban sites in modern times, negating comparisons between urban and non-urban morphology.

Specimens of *Urosaurus ornatus* collected in the 1940's-1960's varied in four characters (Appendix 4). Head width was smaller in park than in mixed forest sites. Proximal and distal forelimb lengths were both smaller in parks than in transitional sites. Distal forelimb length and hindlimb toe length were also smaller in parks than in mixed forest. Specimens of *U. ornatus* from the 1970's-1990's were overwhelmingly represented by a systematic series of collections from 1970-1974 at Hamilton Pool, which is characterized as a mixed forest site. As such, no variation between urban and non-urban sites was observed. The range of values for this decade group, however, is large and includes a significant number of outliers. No morphological variation between sites was found in lizards collected during the 2000's-2010's. Only two modern specimens of *U. ornatus* were captured and measured from a non-urban site, preventing any meaningful morphological analysis.

Specimens of *Cophosaurus texanus* varied widely in urban and non-urban sites (Appendix 5). In the 1940's-1960's, distal hindlimb lengths were smaller in commercial, park, and deciduous forest sites compared to mixed forest and were smaller in parks compared to a university site. Hindlimb toe lengths were smaller in commercial sites than deciduous forest. In the 1970's-1990's, SVL was smaller in deciduous forest than in cropland, but was smaller in cropland than in parks, rangeland, and transitional land. Head length was smaller in cropland than in rangeland, transitional, and park sites. Both forelimb and hindlimb toe lengths were smaller in cropland than in mixed forest and both deciduous forest and transitional sites, respectively. Like *Anolis*, the sample size of earless lizards in the 2000's-2010's was too small to compare morphology (n=3). The sample size for each decade group of *Aspidoscelis gularis* was too small to compare morphology between urban and non-urban sites.

Perch height and type were also analyzed for modern specimens and were not shown to influence morphology. Figure 10 shows regression analyses between perch height and traits for *Sceloporus olivaceus* and *Anolis carolinensis* measured during modern surveys. The *p*-values and adjusted R^2 values

for each regression line are given in Table 4. Figure 11 shows morphological variation between perch type for *Anolis carolinensis,* which was the only lizard with sufficiently different perch types to conduct this analysis. Similar to perch height, there was no variation between perch type in trait measurements.

		Measurement								
	Species	**SVL**	**HL**	**HW**	**PFL**	**DFL**	**PHL**	**DHL**	**FTL**	**HTL**
Perch Height	***Anolis carolinensis***									
	P-Value	0.744	0.465	0.287	0.721	0.384	0.538	0.841	0.054	0.589
	Adjusted R^2	-0.172	-0.067	0.066	-0.167	-0.015	-0.104	-0.189	0.557	-0.15
	Sceloporus olivaceus									
	P-Value	0.727	0.727	0.164	1.43E-01	0.184	0.819	0.661	0.26	0.751
	Adjusted R^2	-0.062	-0.062	0.072	0.093	6.00E-02	-0.072	-6.10E-02	0.03	-6.83E-02

Table 4 – P-values and adjusted R2 values for regression analyses between morphology and perch height for modern specimens of *Anolis carolinensis* and *Sceloporus olivaceus*. Even without Bonferroni correction there is no significant relationship between perch height and morphology based on *p*-values. R2 values were also indicative of a weak relationship between the two, with forelimb toe length in *A. carolinensis* being the strongest indicator of perch height.

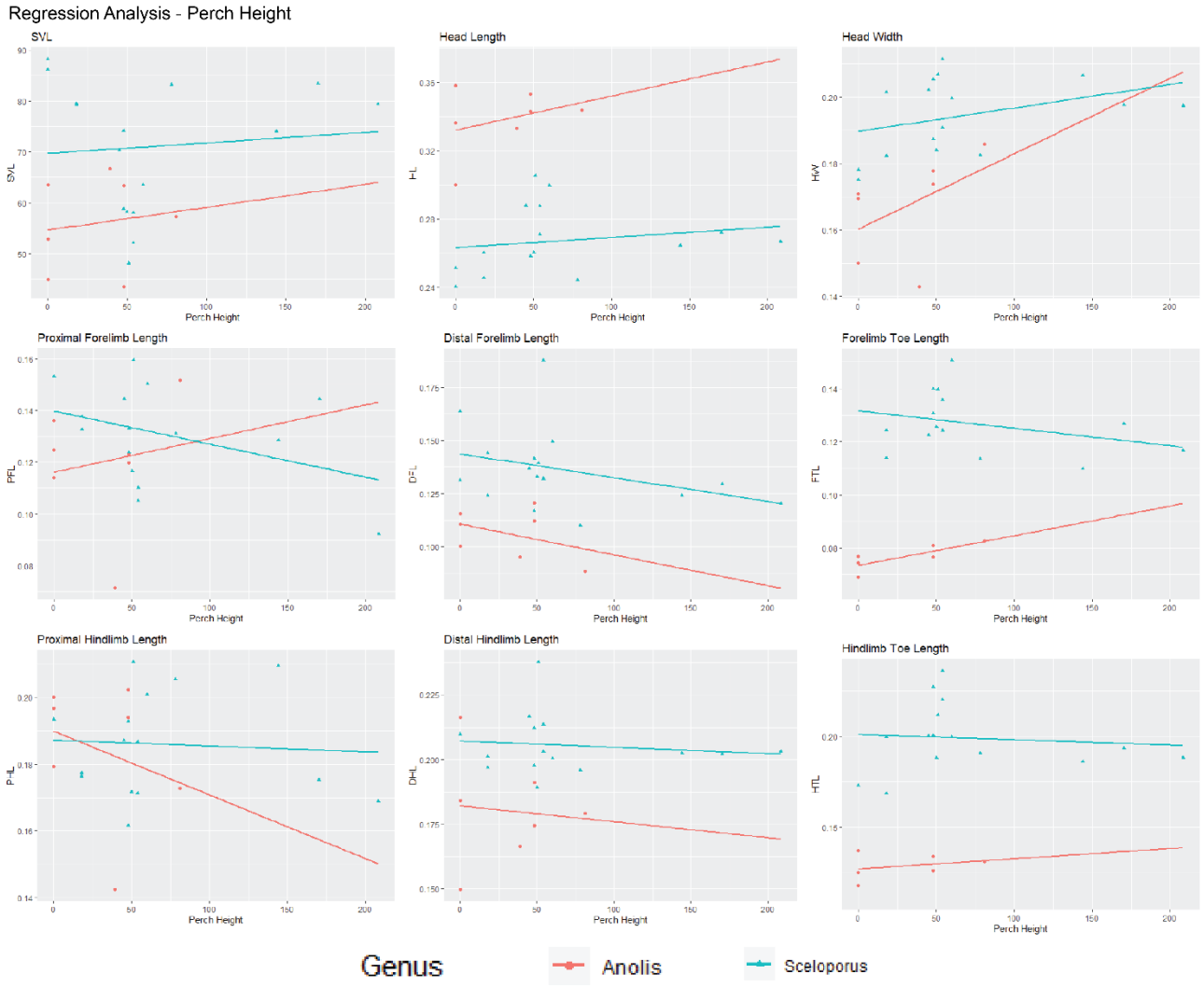


Figure 10 – Regression analysis between perch height and morphological traits for modern specimens of *Sceloporus olivaceus* and *Anolis carolinensis*. *Anolis* is represented by red lines with circular data points, whereas *Sceloporus* is shown by blue lines with triangular data points. There was no significant relationship between perch height and morphology in either species. The regression lines for some measurements in *Sceloporus olivaceus*, such as SVL, head length, proximal and distal hindlimb length, and hindlimb toe length are nearly flat. Additionally, most data points are concentrated towards the left end of the x-axis, indicating perches of less than 100 inches in height.

		Measurement								
Perch Type	Species	SVL	HL	HW	PFL	DFL	PHL	DHL	FTL	HTL
	Anolis carolinensis									
	Tree-Ground	0.283	0.845	0.922	0.143	0.118	0.172	0.558	0.337	0.963
	Tree-Bush	0.141	0.988	0.499	5.58E-02	0.296	0.471	0.965	0.17	0.808
	Ground-Bush	0.86	0.825	0.576	0.883	0.402	0.354	0.508	0.609	0.825

Table 5 – P-values for analysis of morphology and perch type in *Anolis carolinensis* captured during modern surveys in 2021. Values were generated after Kruskal-Wallis and Dunn's test. Even without Bonferroni correction, there was no significant morphological variation between different perch types.

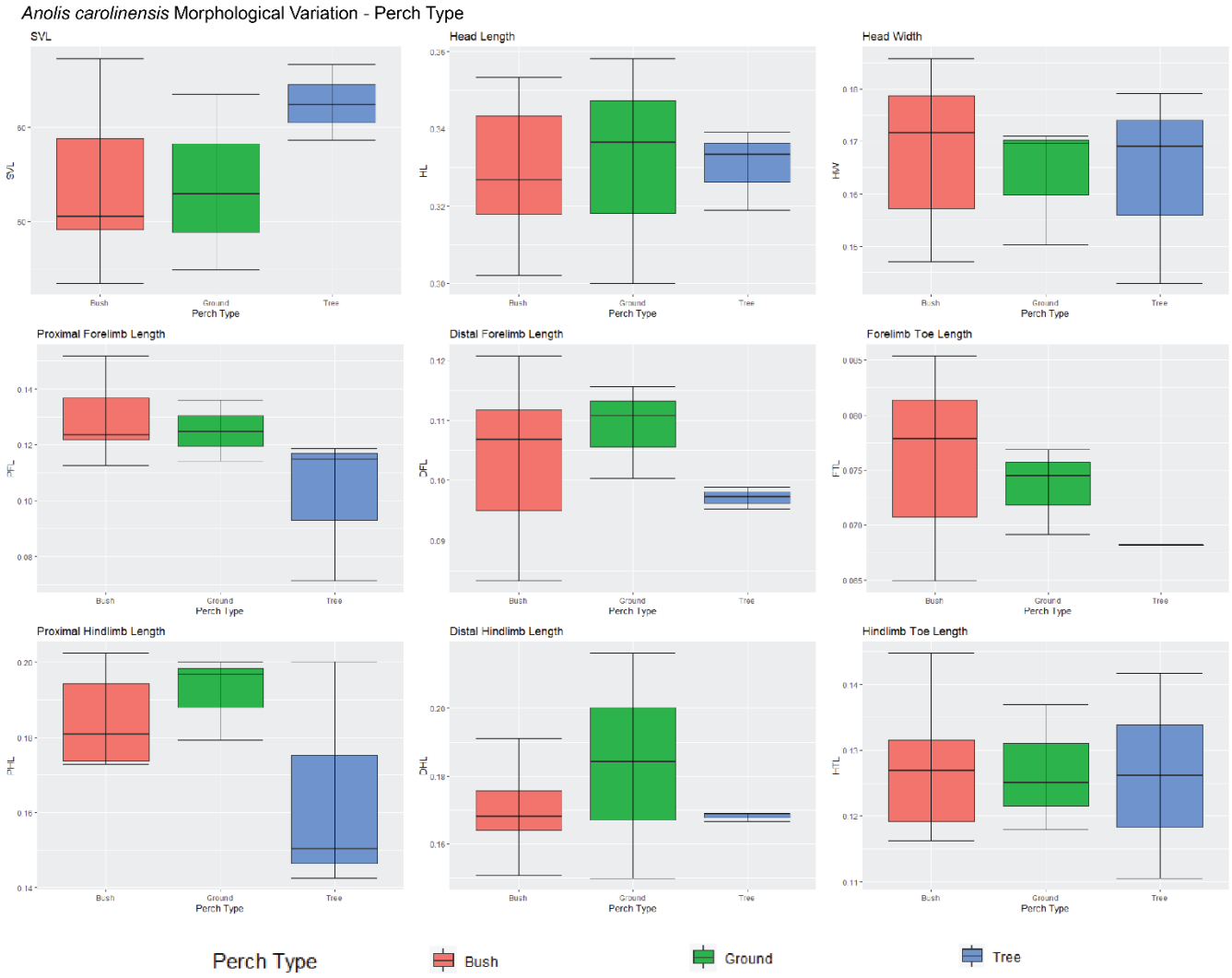


Figure 11 – Morphological variation between perch types in *Anolis carolinensis*. Perch types were not differentiated between urban and non-urban as all lizards were captured in urban environments. Discrepancies in sample size across the perch types, as seen especially in individuals captured on trees, were due to lizards missing toes, limbs, or escaping capture before measurements were completed. There was no significant variation in morphological traits between different perch types after conducting Kruskal-Wallis and Dunn's tests. Sample sizes were also comparable.

Distributions

There were three species with modified distributions: *Sceloporus olivaceus*, *Aspidoscelis gularis,* and *Cophosaurus texanus.* Table 6 shows a presence-absence matrix for all five lizard species at each site during modern sampling efforts, independent of whether specimens were measured. *Sceloporus olivaceus* and *Anolis carolinensis* were the most commonly encountered species, each present in six of the seven

sites sampled. These two species were also the only lizards seen in urban sites whereas *Aspidoscelis gularis* and *Urosaurus ornatus* were only observed in non-urban sites. Of these two, specimens of *A. gularis* were present at all three non-urban sites. *Urosaurus ornatus* was present at St. Edward's Park and Reimer's Ranch, though only specimens at Reimer's Ranch were captured and measured. No specimens of *C. texanus* were observed during modern surveys, despite historical and citizen science records indicating their presence at both the Barton Creek Greenbelt and Reimer's Ranch.

	Site						
	Urban				Rural		
Species	University of Texas	Shoal Creek Park	Oak View Neighborhood Park	Southwest Greenway	Barton Creek Greenbelt	St. Edward's Park	Reimer's Ranch
Anolis carolinensis	1	1	1	1	0	1	1
Aspidoscelis gularis	0	0	0	0	1	1	1
Cophosaurus texanus	0	0	0	0	0	0	0
Sceloporus olivaceus	0	1	1	1	1	1	1
Urosaurus ornatus	0	0	0	0	0	1	1

Table 6 – Presence-absence matrix of all five lizard species during modern surveys. Presence is indicated by a 1, and absence is indicated by a 0. Presence was defined as the direct observation of an individual during field surveys, regardless of whether it was caught and measured. Absence was defined as the lack of any observations of an individual over the entire course of surveys. *Sceloporus olivaceus* and *Anolis carolinensis* were the most commonly encountered species, with each being present at 6 of the 7 sites surveyed. *Aspidoscelis gularis* and *Urosaurus ornatus* were absent from urban sites but were each present at 3 and 1 of the non-urban sites, respectively. *Cophosaurus texanus* was not seen during any modern surveys.

Discussion

Morphology Through Time

I hypothesized that lizards would exhibit morphological variation through time, possibly influenced by urbanization. Overall, I found significant temporal morphological variation in several of the lizard species measured (Tables 2 and 3). Seven out of eight instances of variation in limb measurements indicated a decrease through time. Three out of five instances of variation in toe lengths and four out of five cases of variation in head length and width showed an increase through time. SVL did not have consistent directionality even within species, sometimes decreasing and other times showing an initial decrease followed by an increase in modern times. There is a possibility of sampling bias in the method that historical specimens were collected. Researchers may have collected lizards of a certain size range or chose sites where morphology is not representative of the population as a whole. There could also be differences in trait-to-SVL ratios caused by seasonal plasticity. Male *Urosaurus ornatus* are known to vary in their limb and head lengths depending on the time of year, with measurements increasing in size during the breeding season (Irschick and Meyers 2007). It is therefore difficult to speculate on the mechanisms driving the variation seen. Altered habitat structure or differences in resources driven by urbanization could be important mechanisms that affect many life history traits including microhabitat selection, escape behavior, and resource acquisition (Lambert et al. 2021). These alterations could in turn lead to the morphological variation seen in lizards across time in Central Texas. A more vigorous and systematic study would be required to decipher how urbanization is influencing these morphological differences, and whether it is primarily a plastic response or if increased diversity in phenotypes could be causing microevolution in respective populations. A method to do this would be to begin systematic collections and measurements in modern day, further supported by more accurate land cover data that is now available, to encapsulate the morphological variation of the entire population more fully. Taking

seasonality into account with existing specimens is also valid. My work only utilized specimens at UT Austin; employing specimens from other collections provides a more robust sample and would help better understand these dynamics.

Urban and Non-urban Variation

I also hypothesized that lizard morphology would vary between urban and non-urban environments. Only *Sceloporus olivaceus* from the 1940-1960's showed variation at a general urban-non-urban level; distal hindlimb length was smaller in urban lizards. Most variation between urban and non-urban traits was seen at the most refined spatial level between specific urban and non-urban sites. Out of 72 instances of variation between these refined sites, 32 showed smaller urban measurements than non-urban. Two additional cases showed variation between urban sites where the least urbanized site- park- had larger measurements than more urbanized sites such as commercial and residential. Most studies on urban and non-urban lizard morphology have discovered an opposite pattern whereby limbs and toes are larger in urban settings, affected by different methods and speeds of locomotion needed to capture prey and escape predators and competitors required for different perches (Stuart et al. 2014; Winchell et al. 2018b; Gómez-Benitez et al. 2020). Essentially, changes in habitat structure such as perch type cause lizard limbs to increase in size to allow for better mobility on urban perches such as walls and poles (Winchell et al. 2018b).

A notable exception is a recent study on *Sceloporus occidentalis* in California, whose observations of shorter limbs and toes in urban lizards are consistent with my historical findings (Putman et al. 2019). Interestingly, *Sceloporus olivaceus* measurements did not vary in modern times. However, the only urban sites with *S. olivaceus* were parks which, by their nature, contain more natural perches and uninterrupted habitat for lizards than other more commercial and industrial sites. Consequently, *S. olivaceus* was found utilizing man-made fences with wooden posts that resemble tree trunks (Figure 2). The only urban site

without observed *S. olivaceus* was the University of Texas campus, which is highly paved and has a high density of buildings. These findings suggest that *S. olivaceus* thrive on natural perches such as trees, although the exact process driving this trend is unknown. The use of tree perches across all habitat types could therefore keep urban and non-urban *S. olivaceus* limbs and toes at similar average lengths and negate potential evolutionary and ecological pressures that otherwise affect populations. In contrast, if longer or shorter limb lengths were disadvantageous in highly paved areas, increased urbanization of sites such as the University of Texas may act as a stabilizing selective pressure keeping limb and toe lengths at similar lengths as non-urban lizards. Presently, analysis of perch type and morphology in the only lizard with different perch types, *Anolis carolinensis*, did not show variation (Figure 11). Therefore, more work is needed to disentangle these potentially conflicting processes. Perch height, another variable that could potentially vary between urban and non-urban environments, was also found to not influence morphology (Figure 10). There were eight instances of non-urban averages being smaller than urban averages. Three were found in *Sceloporus olivaceus* (Appendix 2), three in *Anolis carolinensis* (Appendix 3), and two in *Cophosaurus texanus* (Appendix 4). Half of these cases showed lizards from parks with larger measurements. This is further support that the existence of parks that more closely mimic natural habitat and perches may provide a stabilizing force that keeps lizard morphology from being affected by urbanization overall.

One measurement that did not vary much between urban and non-urban environments was SVL, or body size. Only specimens of *C. texanus* collected from the 1970-1990's varied in their body size, and most variation was found between non-urban sites. This could be influenced by environmental variables such as resource availability and habitat structure that become taxon-specific as spatial scale becomes more refined (White et al. 2007). Within the lizard clade, anoles are known to increase in size in urban environments, potentially due to increased resource availability (Thawley et al. 2019), increased predator

abundance (Chejanovski and Kolbe 2019), or other unknown factors. Some of this variation is partially heritable, suggesting evolutionary responses to changing environments (Chejanovski and Kolbe 2019). While this variation was absent in the modern lizard community of Central Texas, continued study could be useful as body size still varied through time and could therefore continue changing as the landscape becomes more urbanized.

While the purpose of this study was not to assess the efficacy of the Bonferroni correction, both its efficacy and shortcomings should be discussed. I applied Bonferroni corrections to temporal analyses to reduce type-I error. However, I excluded them from refined spatial comparisons. Initial application of the correction resulted in no variation between refined urban and non-urban sites in any species within any decade group. Several reviews warn scientists against overzealous use of the correction as a source of type-II error or at the very least urge researchers to be aware of both the method's benefits and pitfalls (Cabin and Mitchell 2000; Armstrong 2014; VanderWeele and Mathur 2019). Since the total number of comparisons for some traits at a refined spatial level would have resulted in a Bonferroni value as high as 25 and a subsequent *p*-value as low as 2.00E-3, I felt this was introducing too much type-II error, especially since variation was found between urban and non-urban morphology at a broader spatial scale.

The contributions of plasticity, evolution, and ecology on morphological variation were the intellectual foundation for all analyses. Urbanization is often suggested as a novel evolutionary pressure on populations, though care must be taken when making such conclusions due to the potentially confounding effects of phenotypic plasticity (Lambert et al. 2021). While my results did not reveal definitive directionality in morphological variation between urban and non-urban environments, the presence of variation itself is notable. The results of my work could be indicative of plastic responses to urban environments that could eventually lead to evolution. Researchers have long sought to understand the evolutionary implications of phenotypic plasticity, with conclusions heavily debated (Wund 2012). In

general, plasticity is shown to potentially allow certain phenotypes to persist in different environments that selection can then act on (Lande 2009). Plastic relationships themselves can also evolve, such as scaling relationships governed by environmental variables (Dreyer et al. 2016). Specifically in lizards, limb size continues to be a highly plastic trait (Irschick and Meyers 2007; Feiner et al. 2020). If urbanization alters lizard morphology in Central Texas through plastic responses, natural selection could potentially act on individuals presenting traits and measurements more suited to urban habitats over time. Looking just at the results of my modern surveys, current urban and non-urban populations of *Sceloporus olivaceus* exhibit similar morphology. In this case, selection would not differentiate between urban and non-urban individuals, and the population would not evolve simply from increased urban development. This is further influenced by ecological mechanisms I did not quantify such as competition and resource acquisition. My distributions analyses, although not extensive and are elaborated on below, suggest that community structure may be altered by urbanization. The filtering of lizards at different sites could then further contribute to which individuals can be acted upon by selection, hindering or driving the evolution of populations.

Distribution Changes

My third hypothesis suggested that lizard distributions changed in Central Texas through time as the region became more urbanized. During my modern field surveys, I noticed the absence of lizards in sites where they occurred historically, either through iNaturalist records or the Biodiversity Collections (Table 6). Specimens of *Sceloporus olivaceus* were collected on multiple separate occasions on the UT campus in the 1940-1960's, but only *Anolis carolinensis* was observed on campus during modern surveys. *Aspidoscelis gularis* was reported in 2021 at the urban Southwest Greenway site but was not noticed during my work (GBif 2021). Finally, although records indicate that *Cophosaurus texanus* exists at the non-urban Barton Creek greenbelt site (GBif 2021), none were found during my surveys.

Notably, lack of presence does not explicitly imply absence. Both *A. gularis* and *C. texanus* are much more terrestrial than the other three lizard species sampled. As such, a different sampling method such as pitfall traps may be useful in capturing these lizards. However, *Aspidoscelis gularis* was found in all non-urban sites and while only a few were captured, they were present in qualitatively high density. This coupled with a lack of observations from Southwest Greenway could be indicative of environmental variables influenced by urbanization causing differences in ecological filtering. The absence of *S. olivaceus* from UT campus is further evidence of a community-wide response to urbanization. Other than available and physical habitat structure, ecological variables that could affect these distribution changes include altered resources such as prey items or altered behaviors stemming from increased human presence. These distribution changes may also be reflected in morphological analysis. The absence of *S. olivaceus* from UT campus may allow *Anolis carolinensis* to utilize previously unavailable resources such as novel microhabitats or take advantage of increased food resources. Over time, this exploitation of novel habitat and resources can affect morphology (Stuart et al. 2014).

As with any study, there were some biases in my sampling efforts that could affect the results of urban and non-urban variation. As mentioned, most urban sites visited were parks. While still urbanized compared to the mixed forest sites visited for non-urban sampling, parks have pockets of uninterrupted habitat and natural perches. These differences from sites with a higher density of human structures such as UT campus could contribute to the lack of variation between urban and non-urban individuals. Non-urban sites visited were also heavily forested, which may reduce open terrestrial habitat for *A. gularis* and *C. texanus*. One site type that I did not visit was agricultural sites. While different than urban environments, agricultural sites are still heavily altered by humans and thus could affect morphology. Agricultural sites should be included in future studies to get a better scope of responses to habitat alteration instead of focusing on forested areas for non-urban sampling.

Additionally, my sampling efforts were limited to using a lasso to capture lizards. About half of *Aspidoscelis gularis* individuals seen evaded capture from the lasso. Diversifying my capture strategies, such as using pitfall traps to capture more terrestrial species such as *A. gularis* and *Cophosaurus texanus*, could have increased my sample size and revealed the presence of lizards at certain sites where they were marked as absent. However, heavily trafficked areas such as parks make the implementation of pitfall traps difficult. I therefore believe my capture strategies were the most prudent and effective for the sites I chose. The timing of my surveys is another potential source of bias. Texas suffered historic low temperatures in early February 2021 that resulted in large parts of Central Texas covered in snow and ice. While lizards were basking and active when I began surveys in late February, lizard populations as a whole may have been affected by the freeze. Species such as *Aspidoscelis gularis* may also be more averse to low temperatures as they were not seen until later in the year.

Future Directions

As mentioned throughout this discussion, many components of this study would benefit from further investigation. Systematic assessments of lizard morphology and distributions between urban and non-urban sites beginning now would serve to provide future researchers with more reliable and complete data. The heritability, or plasticity, of these traits should also be established to begin to accurately discern whether evolution is taking place. This can be coupled with molecular studies to identify the genes contributing to such evolution. Additionally, there were several qualitative observations recorded during modern surveys that warrant further study and connect to greater ecological principles at play. For example, several non-urban *S. olivaceus* basked on the ground by trees and then sprinted up to take cover when approached. In contrast, this behavior was only seen in one urban individual. In fact, most non-urban lizards were more wary when approached, potentially due to less encounters with humans. These behavioral differences could influence limb morphology and subsequent persistence in urban habitats.

Similar behavior has been recorded in *Anolis* and was concluded to be correlated with differences in perches and substrate (Avilés-Rodríguez and Kolbe 2019). *Aspidoscelis* lizards have also been shown to view humans as predators, which could affect escape behavior and locomotion and is in turn affected by morphology (Gómez-Benitez et al. 2020). While my data did not suggest different perch types between urban and non-urban anoles (Figure 11), behavioral differences influence other aspects of lizards' ecology and may have a greater impact as Central Texas continues to become more urbanized. More broadly, work on morphological and other responses to urbanization should be focused on the biological mechanisms governing such responses so as to generalize how organisms respond to this intense anthropogenic pressure. Studies can then become more refined with regards to specific habitats and populations with these processes in mind.

Conclusions

I set out to investigate whether urbanization affects lizard morphology and distributions across spatial and temporal scales in Central Texas. I hypothesized that morphology would vary through time and between urban and non-urban sites, and that lizard distributions changed with increased urbanization. I found temporal variation between urban and non-urban morphology in all five lizard species measured. Specifically, most average limb measurements decreased through time. In *Sceloporus olivaceus*, the lizard species with the largest and most consistent sample size, historical specimens from the 1940's through the 1990's exhibited on average shorter limb and toe measurements in urban sites. Morphological traits in 2021 did not vary between urban and non-urban lizards. While these trends conflict, they are evidence that responses to urbanization are complex and taxon-specific. Additionally, I found no evidence of ecological variables such as perch type and height contributing to morphological differences between urban and non-urban lizards. This further suggests that in modern day, morphology may not be affected solely by urbanization. Finally, I found evidence that lizards are no longer present at sites where they have historical records or are at least present in lower numbers. *Sceloporus olivaceus* was absent from one urban site, and *Aspidoscelis gularis* and *Cophosaurus texanus* were absent from one urban site and one rural site during sampling, respectively. The evidence of morphological variation and distributional changes through time in this part of the Central Texas lizard community suggests that urbanization may continue to have an effect on populations. As such, further research is necessary to elucidate how plasticity, evolution, and ecology will continue to interact with anthropogenic pressures.

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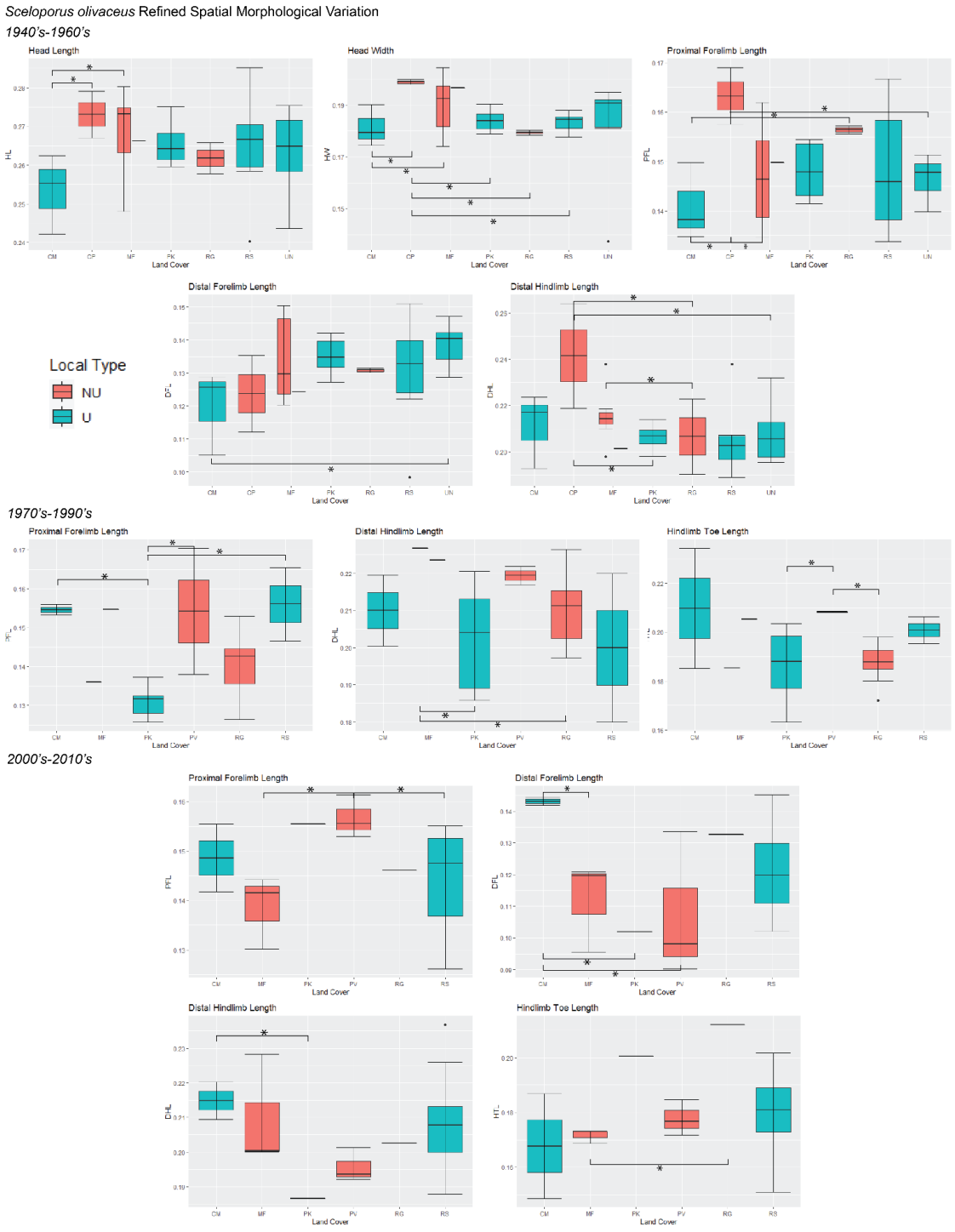
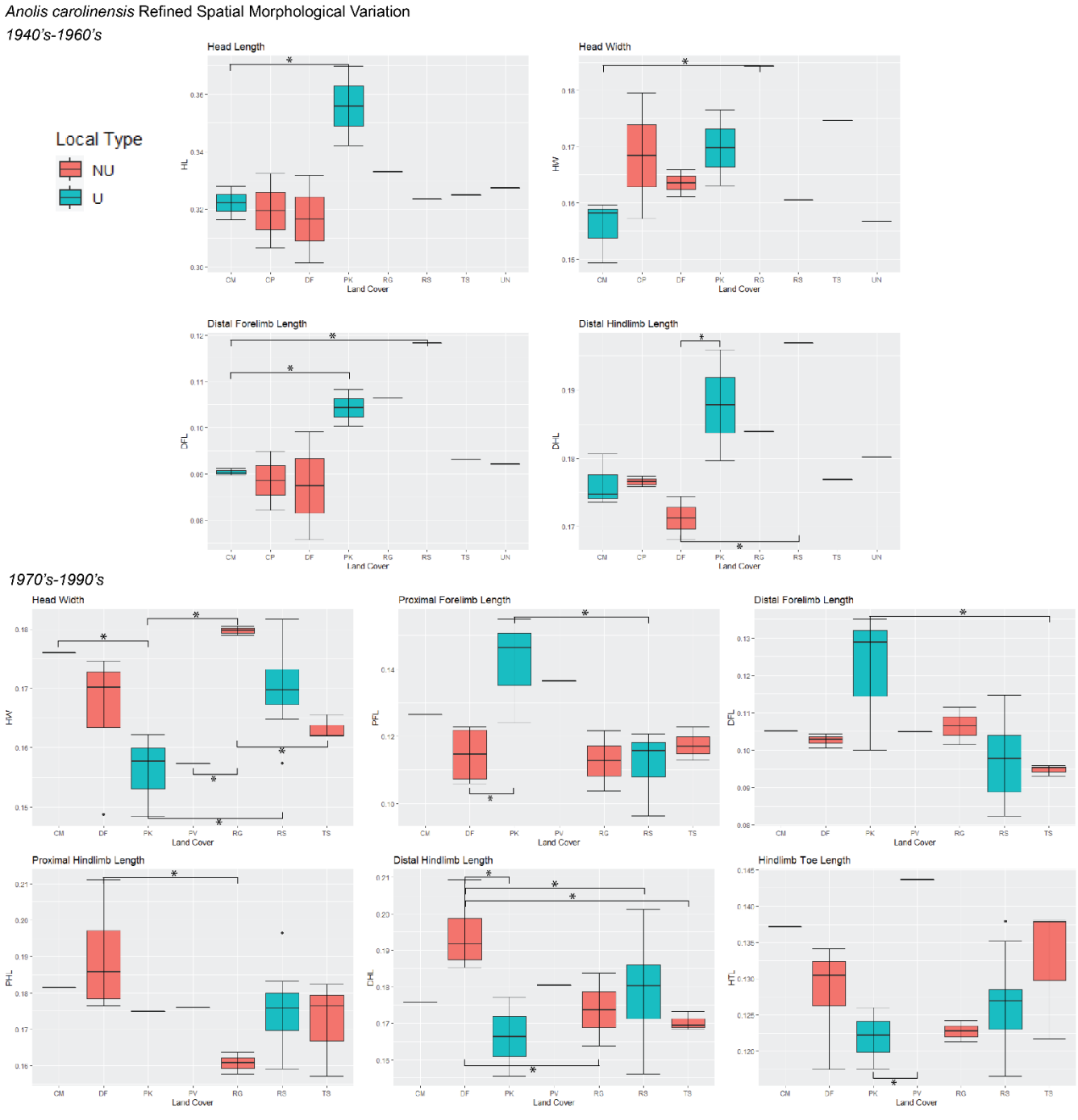
Appendix

Abbreviation	Land Cover Name	Local Type
CM	Commercial	Urban
CP	Cropland	Non-urban
DF	Deciduous forest	Non-urban
MF	Mixed forest	Non-urban
PK	Park	Urban
PV	Preserve	Non-urban
RG	Rangeland	Non-urban
RS	Residential	Urban
TS	Transitional	Non-urban
UN	University	Urban

Appendix 1 – Data dictionary for refined urban and non-urban sites. Refined values were determined using the USGS database (Price et al. 2006).



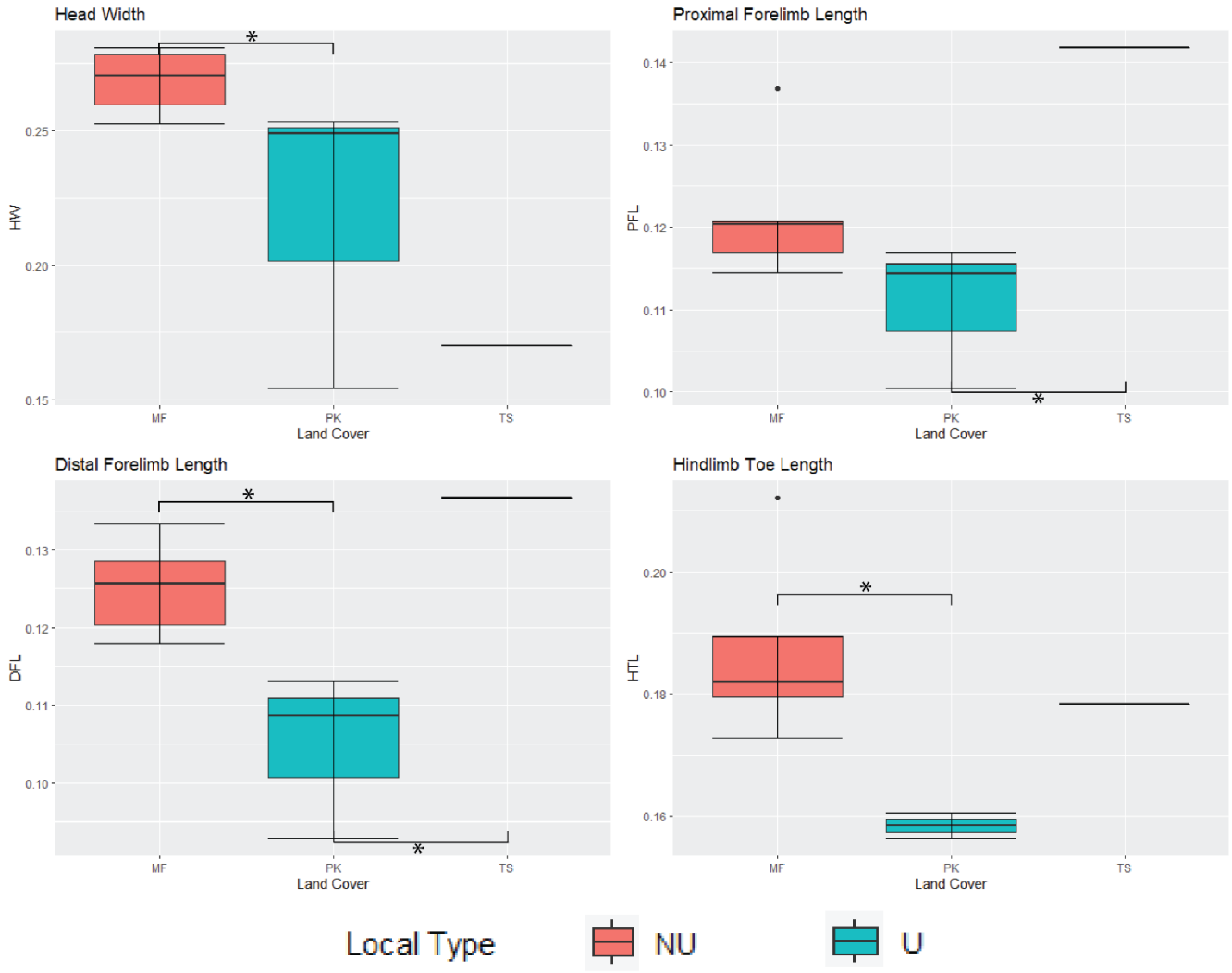
Appendix 2 – Morphological variation between urban and non-urban sites within each decade group for *Sceloporus olivaceus* using refined land cover values. Land cover was determined using categories from the USGS database (Price et al. 2006). Asterisks denote statistical significance determined through Kruskal-Wallis and Dunn's tests and defined as $p<0.05$. Only traits with significant variation are shown. Blue plots indicate urban sites and red plots indicate non-urban sites. Refer to the data dictionary (Appendix 1) for land cover designations.



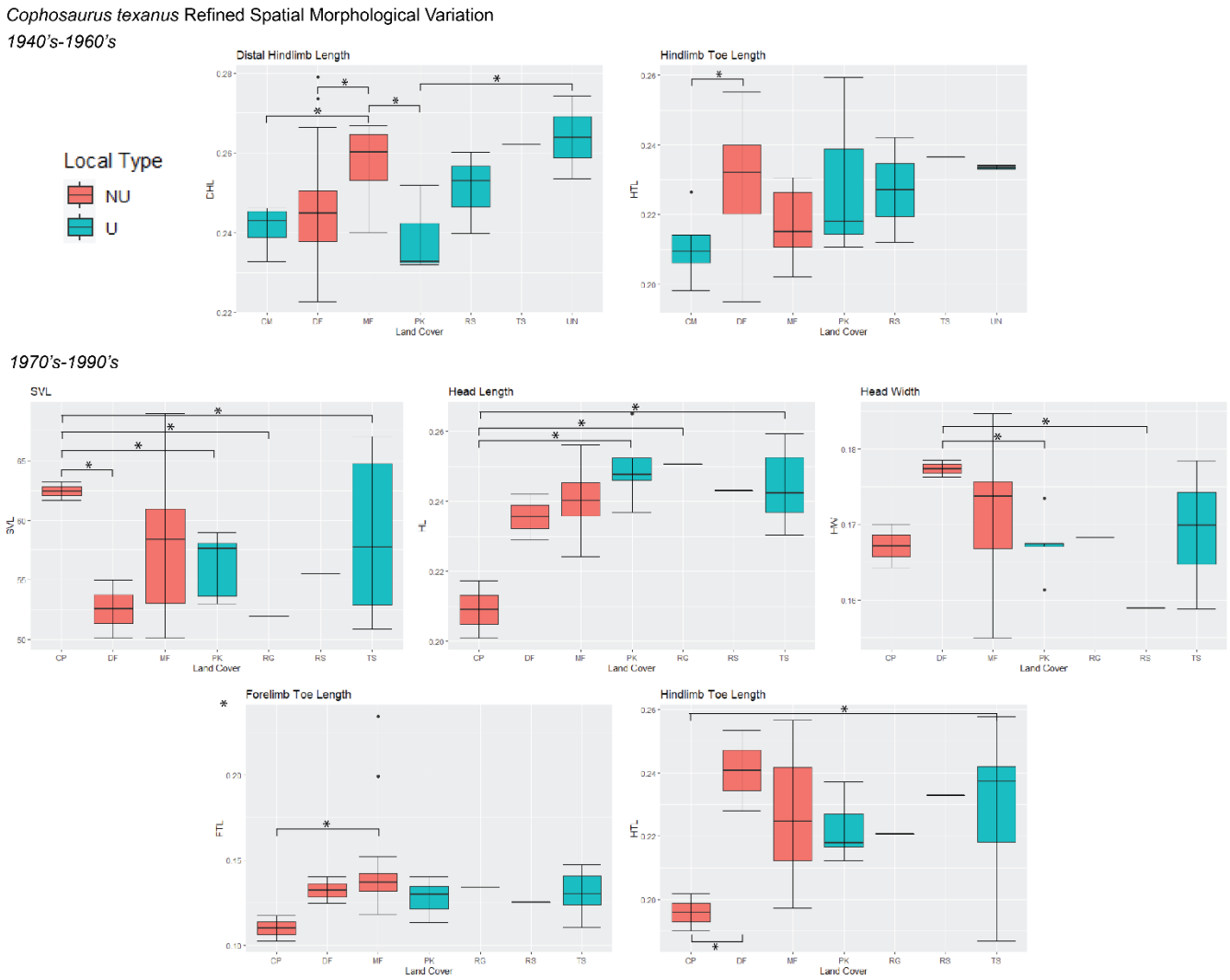
Appendix 3 - Morphological variation between urban and non-urban sites within each decade group for *Anolis carolinensis* using refined land cover values. Land cover was determined using categories from the USGS database (Price et al. 2006). Asterisks denote statistical significance determined through Kruskal-Wallis and Dunn's tests and defined as $p<0.05$. Only traits with significant variation are shown. Blue plots indicate urban sites and red plots indicate non-urban sites. Refer to the data dictionary (Appendix 1) for land cover designations.

Urosaurus ornatus Refined Spatial Morphological Variation

1940's-1960's



Appendix 4 - Morphological variation between urban and non-urban sites within each decade group for *Urosaurus ornatus* using refined land cover values. Land cover was determined using categories from the USGS database (Price et al. 2006). Asterisks denote statistical significance determined through Kruskal-Wallis and Dunn's tests and defined as $p<0.05$. Only traits with significant variation are shown. Blue plots indicate urban sites and red plots indicate non-urban sites. Refer to the data dictionary (Appendix 1) for land cover designations.



Appendix 5 - Morphological variation between urban and non-urban sites within each decade group for *Cophosaurus texanus* using refined land cover values. Land cover was determined using categories from the USGS database (Price et al. 2006). Asterisks denote statistical significance determined through Kruskal-Wallis and Dunn's tests and defined as $p<0.05$. Only traits with significant variation are shown. Blue plots indicate urban sites and red plots indicate non-urban sites. Refer to the data dictionary (Appendix 1) for land cover designations.

Anolis carolinensis		*Aspidoscelis gularis*		*Cophosaurus texanus*			*Sceloporus olivaceus*			
1690	47285	6953		21169	64938	55198	97818	9669	49220	88659
19014	42772	669		50446	61108	55197	9063	6432	49221	68822
1864	42780	13519		1630	64941	97883	97819	71247	49219	
1659	49244	13520		33389	61776	33391	8806	46354	47262	
19013	41743	61107		1688	64935	55196	13163	33453	46566	
57218	49245	41415		5939	47515	64981	9113	33455	41748	
9190	41728	41408		70116	64943	64976	171	6198	41422	
84	41731	41406		70117	64940	64972	97821	4901	89907	
57214	47263	41410		70118	64939	50440	170	97835	92269	
50420	43108	68826		1672	64948	64998	97822	33454	92268	
33355	41860	68827		1674	64934	64970	169	41740	92270	
516	41404	65456		7233	64933	64971	71248	44428	89944	
50421	46576	85893		5940	64932	48437	9157	42058	65579	
42733	47494	68824		1687	64946	64995	9155	46542	65578	
47270	65436	85104		1670	47518	64997	97817	46543	68823	
41756	66573	68825		1673	47521	64996	55203	46633	70052	
42734	89645			1669	64937	64980	13555	46631	70051	
55194	70036			1681	64945	64974	196	46545	97541	
42732	68815			1682	64947	64977	193	46630	88658	
47271				70115	65465	64979	1626	44427	88657	
42794				41017	65464	64978	13006	46544	68668	
47272				1629	65463		9216	46632	68669	
41757				19637	19162		13556	55202	68667	
41858				19161	97882		9197	55201	65056	
43943				100751	55199		964	41228	65057	
41733				5941	57154		1625	65240	68671	
42683				1689	46652		50047	41443	88656	
41730				64931	33393		50050	41421	88655	
41815				47516	1663		50043	42782	88678	
43107				64944	33390		46355	49223	85422	

Urosaurus ornatus

13460	76086	76274	75607	75550	75557	75575	75628	49258	75601	75574
13461	76098	75600	75522	75646	75584	75559	76093	75796	75616	75503
13454	75566	75633	75789	75501	75563	75620	75596	75515	75631	75593
2188	75547	76096	75530	75508	75545	76092	75649	75537	75622	76259
13457	76100	76083	76090	75585	76260	76097	75613	75509	75783	75590
2186	49356	76103	75599	75629	75588	75510	75617	65237	75654	75519
30931	75603	76241	75653	49256	75787	76263	76271	76088	75586	65234
46365	76082	75608	75521	76256	75795	75636	75551	75517	76276	76237
33350	76251	76250	76600	75612	76268	75587	76246	75523	75623	75604
30932	65233	75785	75554	75535	75784	76258	75592	75571	75650	75634
75502	75652	76273	76257	75581	76245	75621	76262	76084	76244	75558
49259	76095	76080	65235	76269	76091	76255	75591	75556	76275	75511
75561	75541	75527	75630	75538	75605	76277	76099	76270	76248	75555
76081	55310	76267	75567	75614	75624	76264	76254	75549	76242	75626
75576	75513	75582	75583	44605	75595	75643	75609	75625	75651	76247

75638	75642	75645	75611	76272
75627	76261	76249	76243	68813
75647	76101	76253	76265	89646
75786	75552	75615	75579	65623
75564	75637	75790	75589	89952
75536	75791	41797	76239	46365
75594	75577	75602	75524	
76252	75640	75560	75648	
76236	75641	75792	76266	
75632	65232	75553	75639	
75793	75780	76240	75644	
75782	75562	75618	75529	
75794	75635	75569	75610	
75580	75520	76238	75619	
75598	75546	76089	75606	

Appendix 6 – Historical specimens measured. All specimens are located at the Biodiversity Collections at the University of Texas at Austin.

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